Ronald J. Nachman[1]
Krzysztof Kaczmarek[1,2]
Howard J. Williams[3]
Geoffrey M. Coast[4]
Janusz Zabrocki[1,2]

[1] *Areawide Pest Management Research Unit, Southern Plains Agricultural Research Center, ARS, U.S. Department of Agriculture, 2881 F/B Rd., College Station, TX 77845*

[2] *Institute of Organic Chemistry, Technical University of Lodz, 90–924 Lodz, Poland*

[3] *Departments of Chemistry and Entomology, Texas A&M University, College Station, TX 77845*

[4] *School of Biological and Chemical Sciences, Birkbeck College, London WC1E 7HX, UK*

An Active Insect Kinin Analog with 4-Aminopyroglutamate, A Novel *cis*-Peptide Bond, Type VI β-Turn Motif

Received 23 June 2004;
accepted 10 August 2004
Published online 29 September 2004 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/bip.20155

Abstract: *The insect kinins are potent diuretic peptides that preferentially form a* cis*-Pro, type VI β-turn. An insect kinin analog containing (*2S,4S*)-4-aminopyroglutamate, a novel* cis*-peptide bond, type VI β-turn motif, demonstrates significant activity in the physiological range in a cricket diuretic assay. This is the first instance of a 4-aminopyroglutamate analog of a peptide with a preference for a type VI turn that demonstrates significant bioactivity. The results provide further confirmatory evidence for the active conformation of the insect kinins, and a new scaffold with which to design biostable, peptidomimetic analogs capable of disrupting critical insect kinin-regulated processes in insects.* © 2004 Wiley Periodicals, Inc.* Biopolymers 75: 412–419, 2004

Keywords: *4-aminopyroglutamic acid;* cis*-peptide bond; β-turn mimetic; constrained insect kinin analog*

INTRODUCTION

The insect kinins share a highly conserved C-terminal pentapeptide sequence Phe-Xaa-Xbb-Trp-Gly-NH_2, where Xaa can be Tyr, His, Ser, or Asn and Xbb can be Ala but is generally Ser or Pro.[1] They have been isolated from a number of insects, including species of Dictyoptera, Lepidoptera, and Orthoptera. The first members of this insect neuropeptide family were isolated on the basis of their ability to stimulate contractions of the

Correspondence to: Ronald J. Nachman; email: nachman@tamu.edu

Biopolymers, Vol. 75, 412–419 (2004)

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JUN 2004		**00-00-2004 to 00-00-2004**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
An Active Insect Kinin Analog with 4-Aminopyroglutamate, A Novel cis-Peptide Bond, Type VI beta-Turn Motif	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **U.S. Department of Agriculture,Areawide Pest Management Research Unit,2881 F/B Rd,College Station,TX,77845**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
see report

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**8**	

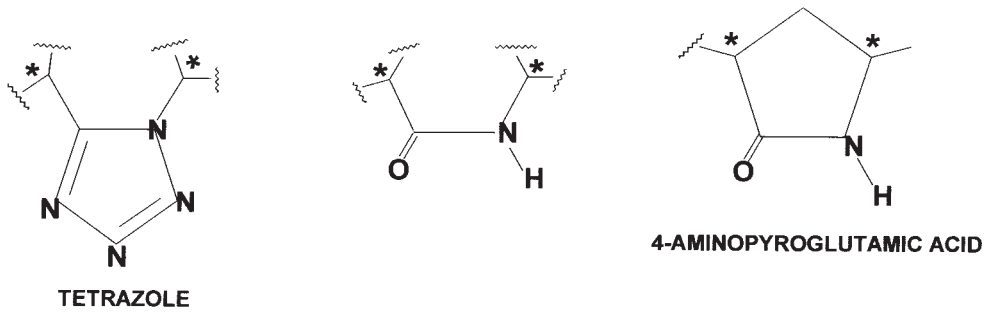

FIGURE 1 A comparison of the structures of the tetrazole ($\psi[CN_4]$, left) and 4-aminopyroglutamic acid (APy; right) motifs, mimics of the *cis*-peptide bond (middle) and a type VI β-turn.

isolated cockroach hindgut,[2,3] but they are also potent diuretic peptides that stimulate the secretion of primary urine by Malpighian tubules, organs involved in the regulation of salt and water balance.[4] In the migratory locust (*Locusta migratoria*) the insect kinins and the corticotropin releasing factor(CRF)-related peptide, colocalized in locust neurosecretory cells, act synergistically to stimulate Malpighian tubule fluid secretion.[4,5] In the housefly, muscakinin has been implicated in the control of diuresis in response to hypovolemia[6] and elicits a four- to fivefold increase in *in vitro* fluid secretion of the Malpighian tubules, more than twice the response observed with the larger CRF-related Musca-DP.[4,5] More recently, insect kinins, and/or analogs, have been reported to inhibit weight gain by larvae of the tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*),[7,8] both serious agricultural pests.

Structurally, the insect kinins require an intact C-terminal pentapeptide sequence for full cockroach myotropic and cricket diuretic activity, which therefore represents the active core.[9] An Ala-replacement analog series of the insect kinin active core region confirms the importance of the Phe and Trp sidechains, because these are the only two replacements that lead to complete loss of myotropic and diuretic activity.[10–12] Due to decreased conformational freedom, active cyclic analogs are more useful for defining the receptor-bound conformation than are linear analogs. Analysis of the conformations adopted by the end-to-end, cyclic insect kinin analog *cyclo*(Ala-Phe-Phe-Pro-Trp-Gly), in which distance and angle constraints obtained from aqueous NMR spectra were incorporated into molecular dynamics (MD) calculations, indicated the presence of two turn types over two distinct sets of residues within the active core pentapeptide. The more rigid of the two conformations featured a *cis*-Pro in the third position of a type-VI β-turn over core residues 1–4, or Phe-Phe-**Pro-**Trp (Figure 1). The other less rigid turn system involved a *trans*-Pro and encompassed residues 2–5, or Phe-**Pro**-Trp-Gly. From unrestrained MD calculations, the most favorable *cis*-Pro structure had an intramolecular energy about 7 kcal/mol lower than the most favorable *trans*-Pro structure, consistent with NMR data that indicated that the *cis*Pro structure was the predominant conformation in solution by a 60:40 ratio.[10–12] This is in agreement with systematic studies on linear peptides with Pro[3] in which the flanking aromatic residues promote the formation of type VI β-turns in aqueous solution.[13]

Although the available evidence (including structure–activity studies) was most supportive of the 1–4 β-turn as the active receptor bound conformation,[10–12,14] the 2–5 β-turn could not be dismissed as a candidate. To obtain more evidence for one of the two available conformations as the active one, an analog containing a tetrazole moiety (Figure 1), which could preferentially form the 1–4 type VI β-turn,[15] was synthesized and found to be active in a cricket diuretic assay in the physiological range.[16] A combination of NMR spectroscopic and computer modeling studies indicated that the tetrazole analog induced a 1–4, type VI turn in aqueous solution and that formation of the alternative 2–5 turn was energetically unfavorable.[16]

Here we report on the synthesis and biological evaluation of an insect kinin analog containing a novel, (*2S,4S*)-4-aminopyroglutamic acid (APy) component (Figure 1) that theoretical and solution conformational studies suggest mimics the *cis*-peptide bond, type VI β-turn in rigid fashion.[17–19] We report the first instance that incorporation of the novel APy component into a peptide sequence whose active conformation has been shown to be a type VI β-turn leads to significant retention of biological activity. The work provides additional confirmatory evidence that this is the active conformation of the insect kinins in the cricket diuretic bioassay system.

MATERIALS AND METHODS

Chemistry

The peptidomimetic analog, Ac-Arg-Phe[b]-(***2S,4S*)-APy**-Trp-Gly-NH_2, was synthesized on an ABI 433A Peptide Synthesizer using modified FastMoc0.25 procedure as well as manually by the solid-phase method, using the Fmoc-strategy and starting from Rink Amide resin (Novabiochem, 0.53 mM/g). The Fmoc protecting group was removed by 20% piperidine in DMF. A fourfold excess of the respective Fmoc-amino acids was activated *in situ* using HBTU (1 Eq)/HOBt (1 Eq) in NMP (automated synthesis) or DCM (manual synthesis) and coupling reactions were base catalyzed with DIPEA (4 equivalents). Amino acid side-chain protecting groups were Pbf for Arg and Boc for Trp. The coupling of Fmoc-(*S,S*)-4-aminopyroglutamic acid (Fmoc-APy-OH) derivative during manual synthesis was mediated by PyBOP (instead of HBTU) in the presence of DIEA in a mixture of NMP and DCM (1:1 v/v). The synthesis of enantiomerically pure Fmoc-APy-OH has been described elsewhere.[20] The completeness of each coupling reaction during manual synthesis was monitored by the Kaiser test. A second coupling was performed when the test was found positive. Cleavage of the peptide from the resin with side-chain deprotection was performed by treatment with trifluoracetic acid (TFA): H_2O:TIS (95.5:2.5:2.5 v/v/v) for 1.5 h. The total volume of the TFA filtrate was reduced to about 1 ml and the peptides were precipitated with cold diethyl ether. The solvents were evaporated under reduced pressure and the resulting materials dissolved in water and lyophilized.

The peptidomimetic analog was purified on a Waters C_{18} Sep Pak cartridge and a δ-Pak C_{18} reverse-phase column (8 × 100 mm, 15 μm particle size, 100 Å pore size) on a Waters 510 HPLC controlled with a Millennium 2010 chromatography manager system (Waters, Milford, MA) with detection at 214 nm at ambient temperature. Solvent A = 0.1% aqueous trifluoroacetic acid (TFA); Solvent B = 80% aqueous acetonitrile containing 0.1% TFA. Conditions: Initial solvent consisting of 20% B was followed by the Waters linear program to 100% B over 40 min; flow rate, 2 ml/min. δ-Pak C-18 retention time: Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2, t_R = 7.5 min. The analog was further purified on a Waters Protein Pak I125 column (7.8 × 300 mm) (Milligen Corp., Milford, MA). Conditions: Flow rate: 2.0 ml/min; Solvent A = 95% acetonitrile made to 0.01% TFA; Solvent B = 50% aqueous acetonitrile made to 0.01% TFA; 100% A isocratic for 4 min, then a linear program to 100% B over 80 min. WatPro retention time: Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2, t_R = 6.0 min. These HPLC conditions have been described in detail elsewhere.[21] Amino acid analysis was carried out under previously reported conditions[21] and used to quantify the peptides and to confirm identity, leading to the following analysis: Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2; G[1.2], F[1.0], R[1.0]. The identity of the peptide analog was confirmed via MALDI-MS on a Kratos Kompact Probe MALDI-MS machine (Kratos Analytical, Ltd., Manchester, UK) with the presence of the following molecular ion (MH^+): Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2, 732.0 [MH^+].

NMR Spectroscopy

NMR spectra were acquired[14,16] on a Bruker ARX-500 500 MHz instrument using a 5 mm Z-gradient Bruker HCN probe. Samples of approximately 1 mM concentration were prepared in 0.5 mL 10% D_2O/H_2O with 0.1 mM DSS as internal standard and were placed in 5 mm × 7 in. Wilmad 535-PP sample tubes. All spectra were acquired in nonspinning mode. Temperature gradient experiments utilized 1D presaturation water suppressed spectra recorded between 5 and 45°C in 5°C intervals using an automated procedure developed in our laboratory utilizing an FTS Systems Air-Jet cooled nitrogen flow set at −20°C as cooling source. Based on N-H temperature shift coefficients, these experiments indicated that no permanent amide hydrogen bonds were present in the analog and all further experiments were performed at 22°C. Two-dimensional (2D) experiments were acquired using 512 increments of 1024 data points, with zero filling to produce 1024 data points in both dimensions. Data were transformed with shifted sine bell window function using the linear prediction forward correction Bruker algorithm. Peak assignments were primarily based on data derived from a TOCSY experiment acquired with a 57-ms mixing period and WATERGATE solvent suppression. Assignments were verified and distance constraints were obtained using data from a ROESY experiment with pulsed 9% duty cycle high power spinlock[1] of 253-ms duration, acquired with water suppression through presaturation. For 2D spectroscopy a 2-s relaxation delay between experiments was used, incorporated in the ROESY experiment as the presaturation period. N-H H-α coupling constants were measured on the 1D spectrum taken at 22°C.

Molecular Modeling

Molecular modeling was performed[14,16] using TRIPOS Sybyl 6.3 software running on SGI Indigo or O2 computers. The peptide was built using the Biopolymers subprogram with the turn promoting analog being prepared by modification of a proline residue. Based on data from the ROESY experiment described above, 13 nontrivial distance constraints were included. Due to computational and time limitations, modeling experiments were performed *in vacuo* with no solvation. No correlations were noted bridging the residues on opposite sides of the APy component. ROESY peak intensity was determined by counting contours. Geminal hydrogen ROESY intensities were used for reference as a strong correlation. Based on these intensities, strong correlations were assigned 1.8–2.7 Å, medium 1.8–3.3 Å, and weak 1.8–5 Å. Distance geometry was used to prepare four unique starting conformations and 200 cycles of simulated annealing were performed on each set. Annealing consisted of heating to 1000°C for 100 ps and then exponential cooling to 200°C over 2000 ps. Annealed conformers were

Table I Diuretic Activity of Peptidomimetic Insect Kinin Analogs in the Cricket *Acheta domesticus*

Peptidomimetic Analog	Stimulation of Malpighian Tubule Fluid Secretion-EC_{50} (10^{-7}M) (% maximal response)
Phe-**Phe**-ψ**[CN_4]-Ala**-Trp-Gly-NH_2	3.4 (100) [95%CL 1.6–7.2][16]
Ac-Arg-Phe-**(2*S*,4*S*)-APy**-Trp-Gly-NH_2	1.4 (93) [95%CL 1.4–1.5]

then energy minimized. The Tripos force field was used as Wiener values are not available for the APy component. Due to computational limitations, experiments were conducted *in vacuo*, with no solvation. Kollman charges were used for normal amino acid components with Gasteiger-Huckel charges for the APy atoms. The three lowest energy conformers from each annealing experiment were compared. Two sets of the four were higher in energy and differed in conformation from the other three, which were similar. The lowest energy form from this set of six was used in further studies. To determine the effects of possible hydrogen bonding, medium constraints based on bonds between all possible amide to backbone carbonyls around the pyroglutamate residue were prepared from the lowest energy form and 50 annealing cycles were performed on these forms. Adding two of the hydrogen bond possibilities as a constraint resulted in forms slightly lower in energy than the nonconstrained models, but, as the temperature gradient study had shown that no permanent hydrogen bonds were present, a dynamics study was performed at low temperature. Finally, the two low energy conformers from this sequence were subjected to 10,000 ps of dynamics with the hydrogen bond constraint removed. The lowest energy conformer from this set was used for the graphics shown. In a further experiment, NMR constraints were removed from the lowest energy conformer and dynamics at 200° for 100,000 fs was performed. This experiment indicated that the area near the APy moiety was fairly rigid but conformational changes occurred more readily as the two termini were approached.

The same low energy form was superimposed on an idealized type VI turn of FFPWG-NH_2 featuring electrostatic interaction between the Phe^1 and Trp^4 aromatic sidechains as had previously observed in other conformationally constrained insect kinin analogs, including *cyclo*[AFFPWG].[10,14,16] Superposition utilized the alpha, beta, and carbonyl carbons of the Phe and Trp residues of the two molecules as well as the Pro nitrogen and Phe-2 carbonyl of the idealized turn model with the nitrogen and carbonyl carbons of the APy ring.

Biology

The diuretic assay has been described in detail elsewhere.[4] In brief, Malpighian tubules were removed from adult female crickets 6 to 12 days old and were transferred to 5 μL drops of bathing solution having the following composition (in mM/L): NaCl, 82; KCl, 27; $CaCl_2$, 2; $MgCl_2$, 8.5; NaH_2PO_4, 4; NaOH, 11; glucose, 24; proline, 10; Hepes, 25. The pH was adjusted to 7.2 with 1 *M* NaOH. The dissected tubules and associated saline droplets are held under liquid paraffin. Urine escapes from a cut made close to the proximal end of the tubule and collects as a discrete droplet in the paraffin. Urine samples are collected at intervals and their volume determined from measurements of droplet diameter under a microscope. After a 40-min equilibration period, the rate of secretion was measured over two 40-min periods before and after the addition of peptide analogs. Diuretic activity is calculated as the increase in fluid secretion (Δ nl/mm/min) and is expressed as a percentage of the response to a supramaximal dose (10 nM) of achetakinin-I assayed on Malpighian tubules taken from the same insect.

RESULTS AND DISCUSSION

Previous evaluation of the insect kinin analog Phe-Phe-ψ[CN_4]-Ala-Trp-Gly-NH_2, incorporating a tetra-

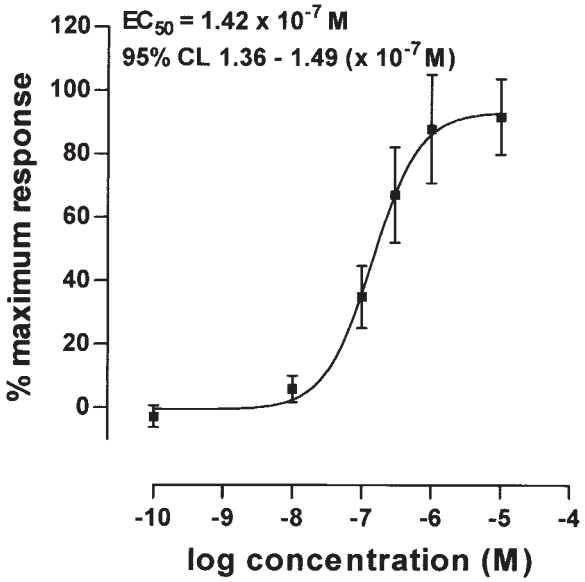

FIGURE 2 Dose–response curve for the insect kinin analog Ac-Arg-Phe-(*2S,4S*)-APy-Trp-Gly-NH_2, containing a novel *cis*-peptide bond mimetic component, in stimulation of Malpighian tubule fluid secretion in the cricket, *Acheta domesticus*.

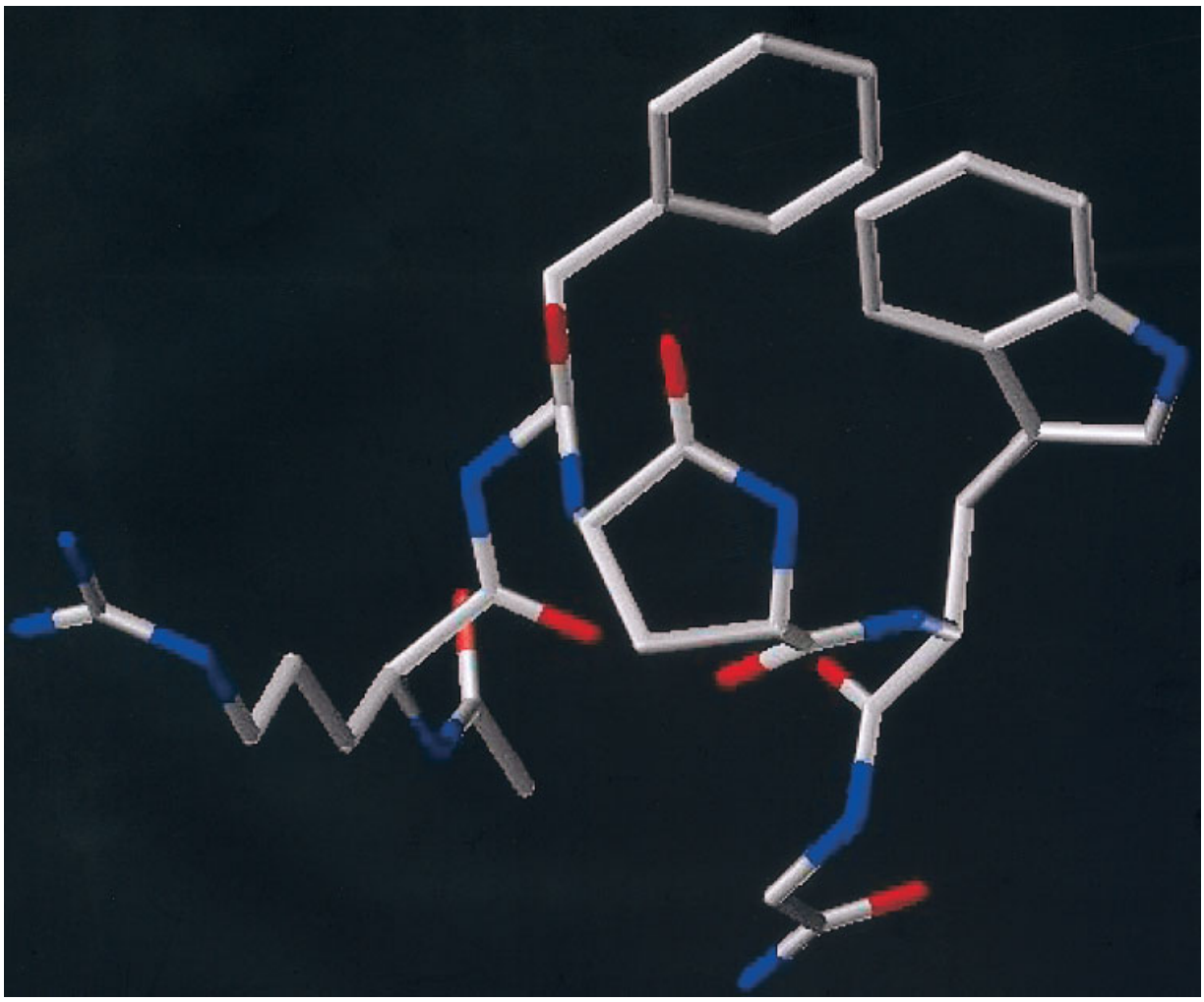

FIGURE 3 Low energy conformation of Ac-Arg-Phe-(***2S,4S***)-**APy**-Trp-Gly-NH_2, incorporating a novel mimic of the *cis*-Pro, type VI β-turn, obtained from a solution conformation study using a combination of NMR spectroscopic data and molecular modeling (see Materials and Methods). The analog demonstrates a preference for an open β-turn and features an electrostatic interaction between the aromatic sidechains of Phe and Trp that brings them into close proximity.

zole moiety (ψ[CN_4]; Figure 1), in a cricket diuretic assay demonstrated significant activity retention in the physiological range, with an EC_{50} of 3.4×10^{-7} *M* and a 100% maximal response (Table I). As the tetrazole is a mimic of a *cis*-peptide bond type VI β-turn,[15–17,22,23] it provided definitive evidence for the active conformation of the insect kinins in the cricket diuretic assay. Using computer modeling data, the 4-aminopyroglutamic acid moiety (Figure 1) has been previously proposed by Paul et al.[17] to mimic the *cis*-peptide bond type VI β-turn in rigid fashion. However, no 4-aminoglutamate analog of a peptide system that evidence suggests preferentially forms the type VI β-turn has as yet been both synthesized and found to retain significant biological activity. Following synthesis of an insect kinin analog incorporating (*2S,4S*)-4-aminopyrogluamic acid (APy) motif, it was evaluated in the cricket diuretic assay and found to demonstrate an EC_{50} of 1.4×10^{-7} *M* and 93% maximal response (Table I; Figure 2), 2.5-fold more potent than the tetrazole analog. The maximal response is not significantly different from that of the tetrazole analog. The (*2S,4S*) stereocehemistry was chosen as it is analogous to *L,L* stereochemical configuration of the α-carbons of the two amino acid residues displaced by the APy moiety in the insect kinin sequence. This therefore represents the first example of an APy peptidomimetic analog that retains significant biological activity.

The solution conformation of the novel insect kinin analog was investigated using a combination of NMR

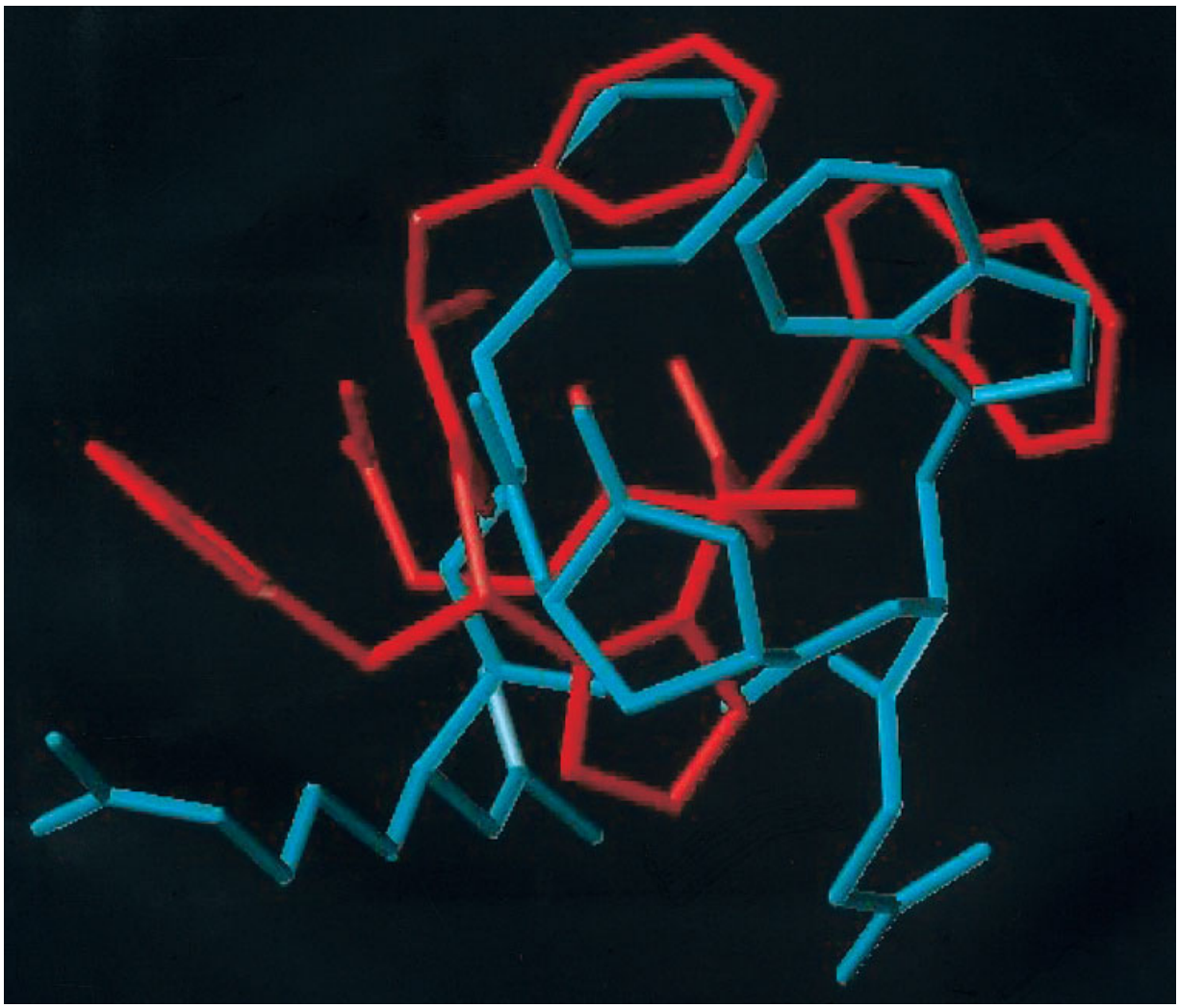

FIGURE 4 Low energy conformations of insect kinin analogs Ac-Arg-Phe[b]-(***2S,4S*)-APy**-Trp-Gly-NH_2 (blue) and Phe-**Phe-ψ[CN_4]-Ala**-Trp-Gly-NH_2 (red), which incorporate *cis*-Pro, type VI mimetic components (APy and tetrazole, respectively), readily superimpose. Both analogs demonstrate a preference for an open 1–4 β-turn, and the Phe-Trp sidechains in both analogs form an electrostatic interaction, leading to a common aromatic surface that is postulated to be an important component of insect kinin receptor interaction.[9,10,14,16]

spectroscopic data and molecular modeling. With no hydrogen bond constraint present, the low energy conformers exhibited an open turn structure with some flexibility. The model indicated fluxional conformations, with only intermittent hydrogen bond formation, although the general turn shape was maintained (Figure 3). An electrostatic interaction between the aromatic sidechains of Phe and Trp is observed, which brings the two aromatic rings into close proximity, as observed in the tetrazole analog and native forms.[9,10,14,16] The conformational studies indicated that the APy analog demonstrated a preference for an open type VI-like β-turn over residues 1–4 and further indicated that formation of the alternate turn over residues 2–5 (featuring a *trans*-Pro in the native kinin sequences) would be energetically unfavorable. In Figure 4, it can be seen that the kinin analogs incorporating an APy and a tetrazole moiety, both mimics[15,17–19] of a *cis*-Pro type VI β-turn, each form very similar open turns that can be readily superimposed upon one another. The significant activity demonstrated by this APy analog provides further confirmatory evidence for the *cis*-Pro, type VI β-turn (Figure 5) as the active conformation of the insect kinin family in the cricket diuretic bioassay. In addition, these results suggest that the APy component, and stereochemical variants, can provide another scaffold for the design of new, biostable agonist and/or antagonist

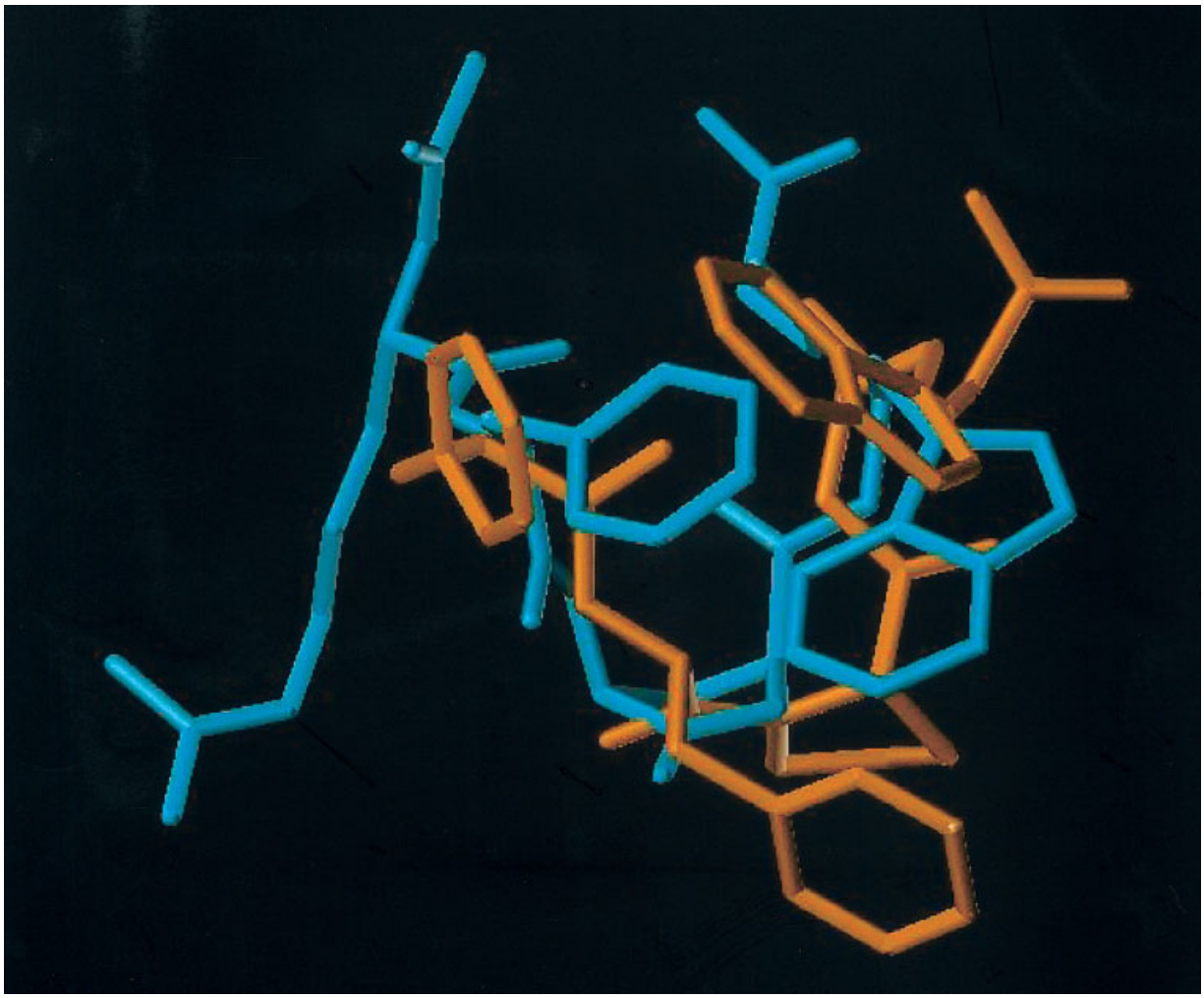

FIGURE 5 Superposition of low energy conformation of the insect kinin analog Ac-Arg-Phe[b]-(***2S,4S***)**-APy**-Trp-Gly-NH_2 (blue) and an idealized type VI β-turn over residues Phe-Phe-Pro-Trp in the insect kinin C-terminal pentapeptide core Phe-Phe-Pro-Trp-Gly-NH_2 (orange) (see Material and Methods). The Phe and Trp sidechains, forming an electrostatic interaction that leads to a common aromatic surface, readily superimpose; as do the *cis* amide bond of the Pro in the insect kinin pentatpeptide and the *cis* amide of the ring in the APy analog.

analogs of this important class of insect neuropeptides. Future research efforts will focus on the synthesis, determination of solution conformation, and biological evaluation of the other three stereochemical APy insect kinin analogs (*2R,4R*), (*2S,4R*) and (*2R,4S*). Biostable, peptidomimetic analogs can potentially disrupt the digestive and diuretic processes regulated by the insect kinins and provide new candidates for future pest insect control agents.

We wish to acknowledge the capable technical support of Allison Strey and Nan Pryor. This work was supported by the U.S. Department of Agriculture (RJN, KK, GMC, JZ), a grant (000517–0103-2001) from the Texas Advanced Technology/Advanced Research Program (HJW, RJN), a Collaborative Research Grant (LST.CLG.979226) from the North Atlantic Treaty Organization (NATO) (RJN, KK, GMC, JZ), and by the State Committee for Scientific Research (KBN, Poland) (KK, JZ).

REFERENCES

1. Holman, G. M.; Nachman, R. J.; Coast, G. M. Peptides 1999, 20, 1–10.
2. Holman, G. M.; Cook, B. J.; Nachman, R. J. Comp Biochem Physiol 1986, 84C, 205–211.
3. Holman, G. M.; Nachman, R. J.; Wright, M. S. In Progress in Comparative Endocrinology; Epple A.;

Scanes, C. G.; Stetson, M. H., Eds.; Wiley-Liss: New York, 1990; pp 35–39.
4. Coast, G. M. Reg Peptides 1995, 57, 283–296.
5. Thompson, K. S.; Rayne, R. C.; Gibbon, C. R.; May, S. T.; Patel, M.; Coast, G. M.; Bacon, J. P. Peptides 1995, 16, 95–104.
6. Coast, G. M. Peptides 2001, 22, 153–160.
7. Seinsche, A.; Dyker, H.; Losel, P.; Backhaus, D.; Scherkenbeck, J. J Insect Physiol 2000, 46, 1423–1431.
8. Nachman, R. J.; Strey, A.; Isaac, E.; Pryor, N.; Lopez, J. D.; Deng, J. G.; Coast, G. M. Peptides 2002, 23, 735–745.
9. Nachman, R. J.; Roberts, V. A.; Holman, G. M.; Tainer, J. A. In Progress in Comparative Endocrinology; Epple, A.; Scanes, C. G.; Stetson, M. H., Eds.; Cambridge University Press: Cambridge, UK, 1990; pp 60–66.
10. Roberts, V. A.; Nachman, R. J.; Coast, G. M.; Hariharan, M.; Chung, J. S.; Holman, G. M. ; Tainer J. J Chem Biol 1997, 4, 105–117.
11. Nachman, R. J.; Holman, G. M.; Coast, G. M. In Recent Advances in Arthropod Endocrinology; Coast, G. M.; Webster, S. G., Eds.; Cambridge University Press: Cambridge, UK, 1998; pp 379–391.
12. Nachman, R. J.; Moyna, G.; Williams, H; Zabrocki, J.; Zadina, J. E.; Coast, G. M.; Vanden Broeck, J. Ann NY Acad Sci, 1999, 897, 388–400.
13. Yao, J.; Feher, V. A.; Espefo, B. E.; Richmond, P. E.; Wright, P. E.; Dyson, H. J. J Mol Biol 1994, 243, 736–753.
14. Moyna, G.; Williams, H. J.; Nachman, R. J.; Scott, A. I. Biopolymers 1999, 49, 403–413.
15. Marshall, G. R.; Humblet, C.; Van Opdenbosch, N.; Zabrocki, J. In Peptides: Synthesis—Structure-Function. Proceedings of the Seventh American Peptide Symposium; Rich, D. H.; Gross, E., Eds.; Pierce Chemical: Rockford, IL, 1981; pp 669–672.
16. Nachman, R. J.; Zabrocki, J.; Olczak, J.; Williams, H. J.; Moyna, G.; Scott, A. I.; Coast, G. M. Peptides 2002, 23, 709–716.
17. Paul, P. K. C.; Burney, P. A.; Campbell, M. M.; Osguthorpe, D. J. Bioorg Med Chem 1992, 2, 141–144.
18. Kaczmarek, K.; Kaleta, M.; Chung, N. N.; Schiller, P. W.; Zabrocki, J. Acta Biochim Polon 2001, 48, 1159–1163.
19. Crisma, M.; Moretto, A.; Toniolo, C.; Kaczmarek, K.; Zabrocki, J. Macromolecules 2001, 34, 5048–5052.
20. Kaczmarek, K.; Chung, N. N.; Schiller, P. W.; Zabrocki, J. In Peptides 2002 (Proceedings of the 27th European Peptide Symposium); Benedetti, E.; Pedone, C., Eds.; Edizioni Ziino: Napoli, Italy, 2002; pp 158–161.
21. Nachman, R. J.; Coast, G. M.; Holman, G. M.; Beier, R.C. Peptides 1995, 16, 809–813.
22. Zabrocki, J.; Smith, G. D.; Dunbar, J. B.; Iijima, H.; Marshall, G. R. J Am Chem Soc 1988, 110, 5875–5880.
23. Zabrocki, J.; Dunbar, J. B.; Marshall, K. W.; Toth, M. V.; Marshall, G. R. J Org Chem 1992, 57, 202–209.

Reviewing Editor: Dr. David Wemmer